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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 2031002PC/ko	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/FI2004/000540	International filing date *(day/month/year)* 15.09.2004	Priority date *(day/month/year)* 15.09.2003
International Patent Classification (IPC) or national classification and IPC C12N15/70		
Applicant FIT BIOTECH OYJ PLC et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 4 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the opinion
   - ☐ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
14.04.2005	20.01.2006
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Scheffzyk, I Telephone No. +49 89 2399-8602

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/FI2004/000540

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-29	as originally filed
Claims, Numbers	
1-36	received on 14.04.2005 with letter of 17.03.2005
Drawings, Figures	
1-26	as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-36
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-12,18-34,36
	No:	Claims	13-17, 35
Industrial applicability (IA)	Yes:	Claims	1-36
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

SECTION V----------

Claims 1-7 and 33 and 34 seem to be novel and inventive since the provision of a selection system as defined in said claims is neither taught nor suggested in the available prior art. Carcinogenesis, vol. 14, no. 2, 1993, Rafael R. Ariza et al., p. 303-305 (1) is considered to represent the closest prior art. This paper describes the use of bacterial strains with a mutation in araD gene in combination with a plasmid carrying an active amber suppressor to provide a method for selection. Presently claimed subject-matter differs from the teaching of (1) in that bacterial cells deficient in araD gene in combination with a vector carrying an araD gene are used as selection system. The use of a vector carrying araD gene in place of the use of a vector containing an active amber suppressor can be seen as inventive since the use of such a vector is neither from (1) nor from any other document cited in the ISR derivable.

As regards claims 8-12 and 36 the presence of an inventive step also can be acknowledged since it was not derivable to a person skilled in the art that the transformation of araD deficient E.coli strains with a plasmid carrying a mutated araD gene with a stop codon at position 8 provides nevertheless said deficient strains with the ability to grow in the presence of L-arabinose (see page 13 of present application).

With respect to claims 13-17 and 35 it is noted that strong doubts exist whether the whole area covered by said claims is actually suitable in presently claimed selection system since from the description it seems to be an essential requirement to the performance of present invention that the plasmid contains an araD gene which results in the production of the corresponding araD gene product, namely L-ribulose-5-posphate 4-epimerase to enable araD deficient strains to survive on media containing L-arabinose. However, vectors containing any mutated araD gene are certainly not suitable for said purpose. Hence, with respect to these claims objections under Art. 5 and 6 PCT arise. Furthermore, an objection for lack of unity may also arise since in so far as the claims under consideration refer to mutated araD genes which are for the above mentioned reasons not deemed appropriate to solve the problem underlying present application they are not necessarily linked to the subject-

matter of the remaining claims. Finally, taking into account that claims 13-17 and 35 cover subject-matter which is apparently not suitable to solve the problem underlying present application, i.e. provision of a selection system, the presence of an inventive step of these claims cannot be acknowledged either.

The same applies to claims 18-32 since the use of E.coli strains deficient in araD gene in selection systems is already taught in (1) (see page 303, right col., third paragraph). Correspondingly, the use of the specific E.coli strains recited in claims 18-32, which may be novel, cannot be considered to be inventive.

Amended Claims as of March 17, 2005

1. A selection system comprising a bacterial cell deficient of an *ara*D gene into which a vector carrying an *ara*D gene, or a catalytically active fragment thereof has been added as a selection marker.

2. A selection system according to claim 1, wherein the *ara*D gene is L-ribulose-5-phosphate 4-epimerase gene (EC 5.1.3.4.).

3. A selection system according to claim 1 or 2, wherein the *ara*D gene is mutated.

4. A selection system according to claim 3, wherein the mutation introduces a stop codon into position 8 of the *ara*D gene.

5. A selection system according to claim 1, wherein the bacterial cell is an *Escherichia coli* cell.

6. A selection system according to claim 5, wherein the *E. coli* is an *E. coli* strain JM109.

7. A selection system according to claim 5, wherein the *E. coli* is an *E. coli* strain DH5 alpha.

8. A vector comprising an mutated *ara*D gene with a stop codon at position 8, or a catalytically active fragment thereof as a selection marker.

9. A vector according to claim 8, wherein the vector is an expression vector comprising:
(a) a DNA sequence encoding a nuclear-anchoring protein operatively linked to a heterologous promoter, said nuclear-anchoring protein comprising (i) a DNA binding domain which binds to a specific DNA sequence, and (ii) a functional domain that binds to a nuclear component, or a functional equivalent thereof; and
(b) a multimerized DNA sequence forming a binding site for the nuclear anchoring protein, wherein said vector lacks a papilloma virus origin of replication, and
(c) the mutated *ara*D gene, or a catalytically active fragment thereof as a selection marker.

10. A vector according to claim 9, wherein the vector is an expression vector comprising:
(a) DNA sequence encoding a nuclear-anchoring protein operatively linked to a heterologous promoter, wherein the nuclear-anchoring protein is the E2 protein of Bovine Papilloma Virus type 1 (BPV), and

(b) a multimerized DNA sequence forming a binding site for the nuclear anchoring protein is of multiple binding sites the BPV E2 protein incorporated into the vector as a cluster, where the sites can be as head-to-tail structures or can be included into the vector by spaced positioning, wherein said vector lacks a papilloma virus origin of replication, and
(c) the mutated *ara*D gene, or a catalytically active fragment thereof as a selection marker.

11. A vector of claim 10 additionally comprising a deletion in the multimerized DNA sequence.

12. A vector of claim 10 additionally comprising a mutation in Shine-Dalgarno sequence.

13. Use of a vector comprising an *ara*D gene, a mutated form of an *ara*D gene, or a catalytically active fragment thereof as a selection marker, in a selection system.

14. Use of a vector according to claim 13 in a selection system, wherein the vector is an expression vector comprising:
(a) a DNA sequence encoding a nuclear-anchoring protein operatively linked to a heterologous promoter, said nuclear-anchoring protein comprising (i) a DNA binding domain which binds to a specific DNA sequence, and (ii) a functional domain that binds to a nuclear component, or a functional equivalent thereof; and
(b) a multimerized DNA sequence forming a binding site for the nuclear anchoring protein, wherein said vector lacks a papilloma virus origin of replication, and
(c) the *ara*D gene, a mutated form of an *ara*D gene, or a catalytically active fragment thereof as a selection marker.

15. Use of a vector according to claim 14 in a selection system, wherein the vector is an expression vector comprising:
(a) DNA sequence encoding a nuclear-anchoring protein operatively linked to a heterologous promoter, wherein the nuclear-anchoring protein is the E2 protein of Bovine Papilloma Virus type 1 (BPV), and
(b) a multimerized DNA sequence forming a binding site for the nuclear anchoring protein is of multiple binding sites the BPV E2 protein incorporated into the vector as a cluster, where the sites can be as head-to-tail structures or can be included into the vector by spaced positioning, wherein said vector lacks a papilloma virus origin of replication, and
(c) an *ara*D gene, a mutated form of an *ara*D gene, a complementary sequence thereof, or a catalytically active fragment thereof as a selection marker.

16. Use of a vector of claim 15 in a selection system, wherein the vector additionally comprises a deletion in the multimerized DNA sequence.

17. Use of a vector of claim 15 in a selection system, wherein the vector additionally comprises a mutation in Shine-Dalgarno sequence.

18. Use of *E. coli* strain AG1 deficient of the *ara*D gene in a selection system.

19. Use of *E. coli* strain JM109 deficient of the *ara*D gene in a selection system.

20. Use of *E. coli* strain DH5alpha-T1 deficient of the *ara*D gene in a selection system.

21. *E. coli* strain DH5alpha-T1 deficient of the *araD gene and ulaF* gene.

22. *E. coli* strain DH5alpha-T1 deficient of the *ara*D gene and sgbE gene.

23. *E. coli* strain DH5alpha-T1 deficient of the *ara*D gene, *ulaF* gene, and sgbE gene.

24. *E. coli* strain AG1 deficient of the *araD gene and ulaF* gene.

25. *E. coli* strain AG1 deficient of the *ara*D gene and sgbE gene.

26. *E. coli* strain AG1 deficient of the *ara*D gene, *ulaF* gene, and *sgb*E gene.

27. Use of *E. coli* strain DH5alpha-T1 deficient of the *araD gene and ulaF* gene in a selection system.

28. Use of *E. coli* strain DH5alpha-T1 deficient of the *ara*D gene and sgbE gene in a selection system.

29. Use of *E. coli* strain DH5alpha-T1 deficient of the *ara*D gene, *ulaF* gene, and sgbE gene in a selection system.

30. Use of *E. coli* strain AG1 deficient of the *araD gene and ulaF* gene in a selection system.

31. Use of *E. coli* strain AG1 deficient of the *ara*D gene and sgbE gene in a selection system.

32. Use of *E. coli* strain AG1 deficient of the *ara*D gene, *ulaF* gene, and *sgb*E gene in a selection system.

33. A method of selecting the cells transformed with a plasmid containing an *ara*D gene, or a catalytically active fragment thereof as a selection marker and the gene of interest, the method comprising inserting the plasmid into the *ara*D deficient host cell and growing the cells in a growth medium containing arabinose.

34. A method of claim 33 wherein the *ara*D gene is L-ribulose-5-phosphate 4-epimerase gene (EC 5.1.3.4.).

35. A method of claim 33 or 34, wherein the *ara*D gene is mutated.

36. A method of claim 35, wherein the mutation introduces a stop codon into position 8 of the *ara*D gene.